OBSERVATION ON THE ECOLOGY AND REPRODUCTION OF FREE-LIVING CONCHOCELIS OF PORPHYRA TENERA

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In a previous paper (Iwasaki, 1961) it was shown that free-living *Conchocelis* colonies produce normal monosporangia and monospores under short-day conditions (8–11 hours light daily); these monospores germinate into leafy thalli as they do in nature.

The free-living *Conchocelis* colonies cultured in continuous light produce only other *Conchocelis* colonies. It became then interesting to find how new *Conchocelis* colonies are produced. It was found that new colonies could be obtained from small pieces of *Conchocelis* filaments. However, the *Conchocelis* colonies grown in continuous light produce sporangia which are morphologically different from the normal sporangia produced under short-day conditions. Even though free spores were not found in continuous light, it was not excluded that new *Conchocelis* colonies could be produced by special spores produced under continuous light.

The present work was undertaken to study the type of sporangia formed by free-living *Conchocelis* colonies under long-day conditions, and the fate of their spores. It was found that spores are indeed formed and that they can originate new *Conchocelis* colonies, revealing, under these conditions, a new part of the life-cycle of *P. tenera*.

Materials and Methods

The original material derives from mature leafy thalli collected at Matsukawa-ura Inlet located near Sendai in 1960. The *Conchocelis* colonies used for the present studies were obtained from carpospores produced by the leafy thallus passing through one life-cycle *in vitro*. This *Conchocelis* strain was a uni-algal culture, but accompanied by bacteria and yeast. Two treatments with ultraviolet light for 1–2 minutes, at intervals of one month, eliminated the bacteria but not the yeast. Two types of liquid media, the artificial medium ASP12NTA (Provasoli's medium), and the enriched sea water medium SWI, were employed for the experiments (Table I). The cultures were carried on mainly in screw-cap tubes (125 × 20 mm.) with 10 ml. of medium. When necessary, the hanging culture technique was employed.

Results

I. *Types of sporangia produced in long-day conditions*

a) The "inflated spherical" cells

Small pieces of *Conchocelis* filament were inoculated in culture media and grown under different conditions. The first observation was made on the strain cultured at a window in subdued natural light (max. 500 ft.c.) from July to

TABLE I

Enriched sea water medium, SWI

Filtered sea water	1000 ml.
KNO_3	72.2 mg.
KH_2PO_4	8.8 mg.
Fe-EDTA (1:1 chelation)	0.5 mg. (as Fe).
"Tris Buffer"*	500 mg.
pH	7.8–8.0

* Tris (hydroxymethyl) amino methane (Sigma Company).

Artificial medium ASP12NTA

Distilled water	100 ml.	$Na_2SiO_3 \cdot 9H_2O$	15 mg.
NaCl	2.8 g.	B_{12}	0.02 μg.
$MgSO_4 \cdot 7H_2O$	0.7 g.	Biotin	0.1 μg.
$MgCl_2 \cdot 6H_2O$	0.4 g.	Thiamine	10 μg.
KCl	0.07 g.	P II metals*	1 ml.
Ca (as Cl)	40 mg.	S II metals**	1 ml.
$NaNO_3$	10 mg.	"Tris" buffer	0.1 g.
K_3PO_4	1 mg.	Nitrilotriacetic acid	10 mg.
Na_2 glycerophosphate	1 mg.	pH	7.8–8.0

* One ml. of P II metals contains: EDTA, 1 mg.; Fe (as Cl), 0.01 mg.; B (as H_3BO_3), 0.2 mg.; Mn (as Cl), 0.04 mg.; Zn (as Cl), 0.005 mg.; Co (as Cl), 0.001 mg.

** One ml. of S II metals contains: Br (as Na), 1.0 mg.; Sr (as Cl), 0.2 mg.; Rb (as Cl), 0.02 mg.; Li (as Cl), 0.02 mg.; I (as K), 0.001 mg.; Mo (as Na), 0.05 mg.

September at temperatures ranging from 20 to 28° C. Small plants of *Conchocelis* formed new lateral branches in a week. Some of the tips of branches swelled to form a more or less spherical cell about three weeks after inoculation. These cells became more deeply pigmented and gradually reached 11.4–14.0 μ in diameter (Plate I, A). Soon the spherical cells detached from the branches and became free spores, mostly spherical in shape (9.8–11.7 μ in diameter, Plate I, B). The spores begin to germinate after 3–7 days from the liberation. At first the spore forms a germ tube, then a delicate cross-wall appears between the original spore and the tube, and soon lateral branches form. These early filamentous germlings were usually quite tortuous. They did not orient to any particular direction in relation to light. Finally, the germlings grew into luxuriant branched filamentous thalli.

b) The special "sporangia"

In the culture grown at 17–19° C. and exposed to 16 hours fluorescent light (350 ft.c.), the *Conchocelis* colonies produced many sporangia (SI) as shown in Plate I, D–F. The sporangia are very similar to the sporangia produced under continuous light (Iwasaki, 1961, Fig. 4, p. 178). As mentioned in that paper, the sporangia produced under long-day conditions seemed to be different morphologically from the monosporangia produced under short-day conditions. The sporangia cells have thicker walls and the length of cells is usually about half their diameter.

c) The "strawberry-like" bodies

In the mass culture under the same conditions, very strange, round strawberry-like structures (70–80 μ in diameter) were found. These structures (Plate I, C)

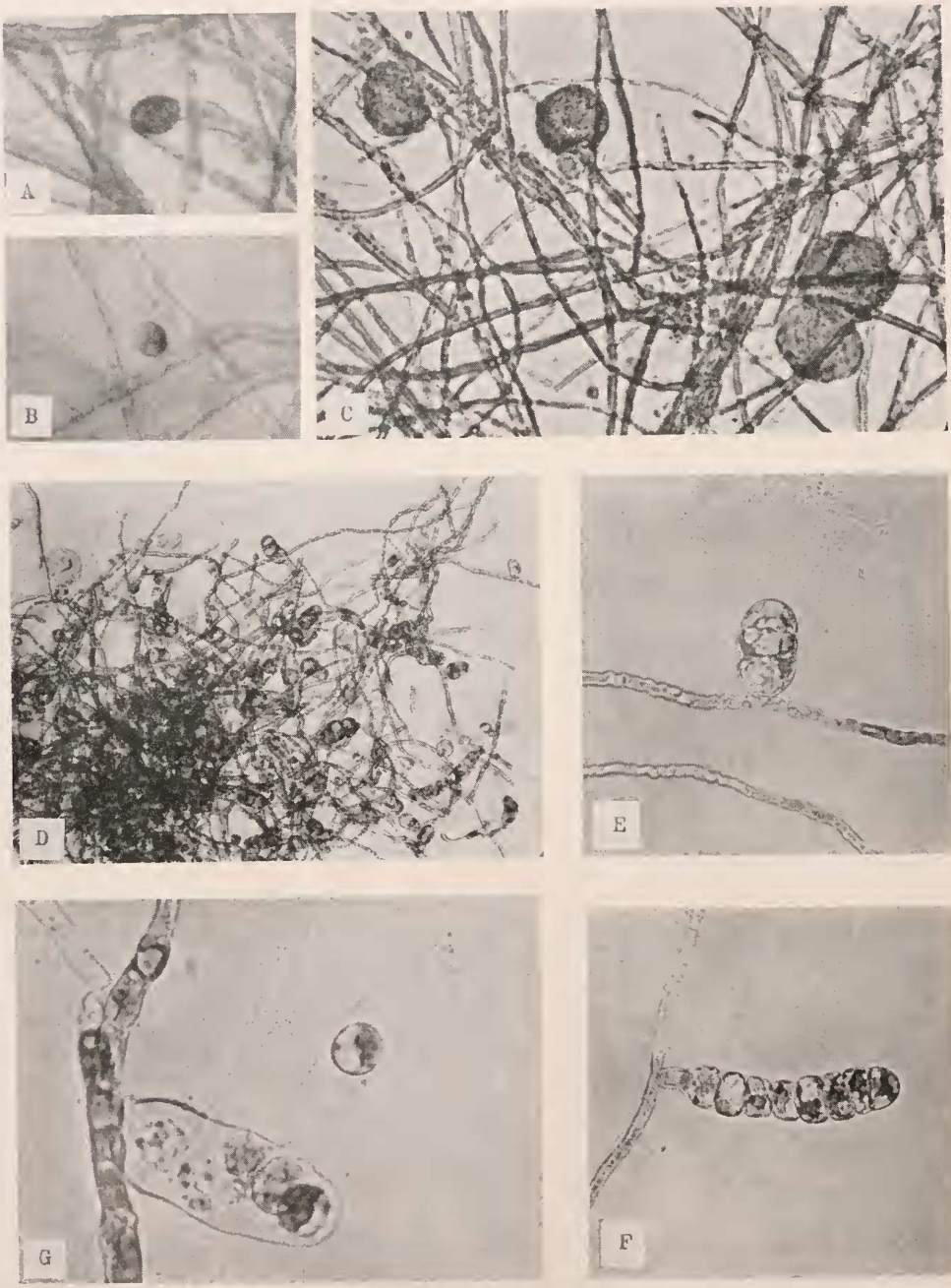

PLATE 1

A, B. Inflated spherical cells (A) and liberated concho-monospore which germinate into *Conchocelis* thallus (B). × 256. C. Round strawberry-like structures formed on the *Conchocelis* branches. × 160. D, E, F, G. Sporangia (SI) formed in long-day conditions; D, × 96; E, × 192; F, × 160; G, liberation of the spores, × 384.

have numerous very slender colorless filaments, less than 1 μ broad, 10–20 μ in length, on the surface. At first they seemed to be some kind of parasitic fungi, but about a month after their appearance, many new filamentous *Conchocelis* colonies were found in the culture.

II. *Fate of the "spores" produced by the "strawberry-like" bodies*

Suspecting that these germlings might derive from the "strawberry-like" bodies, 16 of these structures were cut off with a scalpel from the branches, then cultured in hanging drops (ten structures) and in screw-cap tubes (six structures), respectively, under identical conditions (long day, 350 ft.c. fluorescent light). These structures, even after the separation from the branches, grew to the size of about 140 μ in diameter.

a-1) In two cultures in hanging drops, several germ tubes grew directly from the strawberry-like structure after 16 days. The germ tubes elongated, formed lateral branches, and then grew into *Conchocelis* colonies by repeated branching.

a-2) In two other cultures, after three weeks or more, the structures disintegrated, liberating several ameboid, unicellular spore-like bodies. Two of them germinated in a way similar to the carpospores produced by leafy thallus and grew into free-living *Conchocelis* colonies. Two kinds of *Conchocelis* filaments were observed, one thick (7.6–8.0 μ broad) and the other thin (4.0–5.0 μ broad). These two kinds of young *Conchocelis* were transferred to new medium, but they became infected by bacteria and the culture medium became cloudy. The thick-filamented *Conchocelis* grew nicely despite the bacterial infection and the culture medium became clear after three weeks. The thin-filamented *Conchocelis* bleached soon after transfer.

a-3) The other strawberry-like structures in hanging drops did not germinate and became covered by very slender dark brown filaments after a month or more.

b-1) The six structures inoculated in screw-cap tubes attached themselves immediately to the side wall of the tubes. After two weeks the internal cells of the structure produced several germ tubes which grew into normal *Conchocelis* (four cultures). One of these *Conchocelis* colonies formed monosporangia after 45 days, and soon liberated monospores that gave rise to leafy thalli reaching 2–3 mm. before they became pale and died under the same long-day conditions. Half of these monospores were isolated and also germinated into normal leafy thalli under short-day conditions (9 hours daily of incandescent light) at 13–15° C. Three other *Conchocelis* cultures formed very poor sporangia which did not liberate any spores, either under long-day or under short-day conditions (150–300 ft.c. fluorescent light for 270 days).

b-2) One of the strawberry-like structures disintegrated in two weeks but after a month, eight young *Conchocelis* colonies were found. Unfortunately, the observation of this experiment was done through the wall of the test tube, using a dissecting microscope, so the germination could not be followed in detail. It is probable that these young *Conchocelis* colonies originated from ameboid-shaped spores produced by the strawberry-like structure. These *Conchocelis* colonies formed many monosporangia and liberated a lot of monospores under short-day conditions. These monospores also germinated into normal leafy thalli.

III. *Fate of the "spores" of long-day sporangia (SI) under different light periods*

Half of the *Conchocelis* culture, which formed "parasporangia-type" sporangia (SI, see section Ib), were moved to short-day conditions (9 hours daily of 200 ft.c. incandescent light) at 13–15° C., and the other half were kept at 17–19° C. under fluorescent light of 200 ft.c. and a daily photoperiod of 14 hours. In about two weeks, the sporangia discharged many spores. The liberation of the spores was a little earlier in the short-day condition. The manner of spore liberation is shown in Plate I, G. These spores were taken up with a capillary pipette and inoculated in new culture media. The majority of spores did not germinate either under short-day or long-day conditions. In general a distinctly higher percentage of germination was observed in closely grouped spores than in less closely grouped clusters. Many abnormal pale germlings (Plate II, E, F), which soon bleached, were observed.

a) Under long-day conditions the spores gave rise to filaments but soon the original spore enlarged and divided, forming a sporangia-like body (Plate II, A). Sporangia-like bodies are also formed in the terminal part of filaments or as lateral growth on the singly-branched filaments (Plate II, B). After that the filaments became *Conchocelis* colonies and produced well developed luxuriant sporangia-like bodies (Plate II, B).

Some other spores continue to enlarge after liberation; occasionally they produce root-like projections and grow into globular bodies, about 110 μ in diameter (Plate II, C, D). Another group of spores enlarged, then grew into irregular-shaped "blades" as shown in Plate II, G.

b) The spores kept under short-day conditions produce short, colorless filamentous projections (about less than 100 μ). The projections may occasionally branch. The original spore enlarged while the filamentous projections elongated and became more deeply pigmented, then formed a cross-wall (Plate III, F). At this stage, the filamentous part bleached while the original spore continued to divide, forming a sporangia-like body composed of cells quite short and broad (Plate III, G). No more elongation and longitudinal division of germlings occurred even under these conditions which are suitable for the growth of leafy thallus. Some germlings produce robust branches near the original spore body, occasionally on the first sporangia-like branch, and grow into the aberrant plantlets as shown in Plate III, A–E. "Massive plantlets" (Plate III, I–K, L–O), irregular-shaped blades and abnormal germlings, as mentioned above, were also found in the culture under short-day condition.

Discussion

New *Conchocelis* colonies can grow from small pieces of filaments (less than 1 mm. in length). Some of the new colonies, therefore, may derive from small pieces of filaments that are cut off naturally, especially in old cultures, and by shaking.

Inflated spherical cells are often formed at the tip of branches. These inflated cells were at first thought to be undeveloped monosporangia, but no leafy thalli developed from the spores found in these cultures. It is sure, therefore, that the spores derived from inflated cells are quite different functionally from the monospores that develop into leafy thallus. It is not clear yet whether or not the inflated cells are formed on definite branches. This asexual reproduction closely resembles

PLATE II

Various germlings developed from "spores" originating from sporangia (S1) in 14 hours' illumination daily. A, B, *Conchocelis*-like plants; A, × 160, B, × 72. C, D, globular body, × 160. E, F, (Abnormal one?) × 160. G. Blade-like body, × 160.

that of *Rhodochorton*. It would seem fitting to call them "Concho-monospores" to specify that they originate new *Conchocelis* colonies and to differentiate them from the normal monospores which develop into leafy thalli.

It is not clear whether the strawberry-like structures are polysporangia or cystocarps. The difference observed in the germinative processes derived from isolated strawberry-like structures seems mainly due to the maturation stage of the structure. The strawberry-like structures matured normally on the *Conchocelis* branches, liberating two to eight spores which germinated into *Conchocelis* colonies.

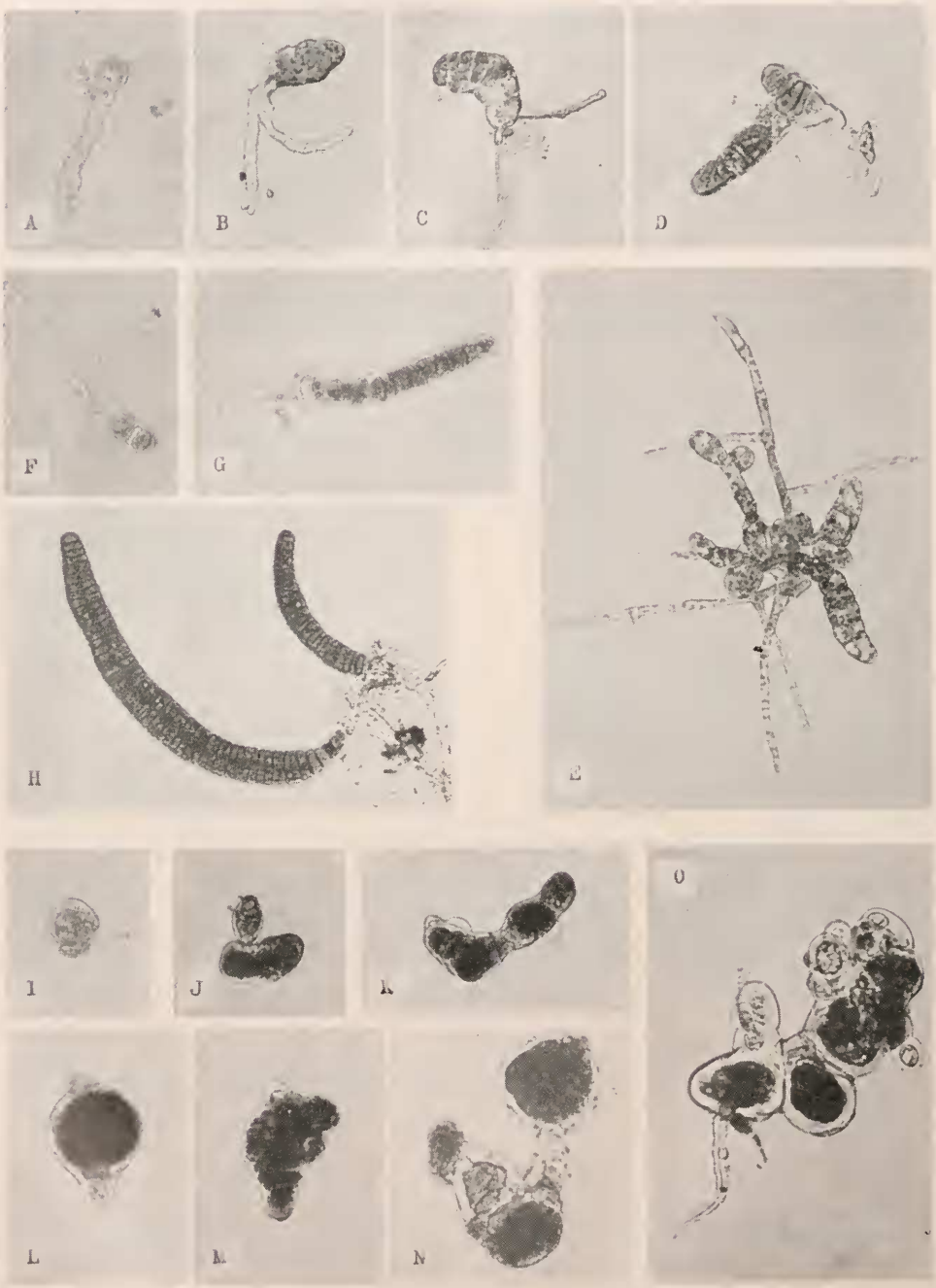

PLATE III

Various germlings developed from "spores" originating from sporangia (SI) in 9 hours' illumination daily (except H). A–E, Plantlets developing from the "spores," × 160. E, × 192. F–G, Blade-like bodies, × 160. I–O, Massive plantlets, × 160. H, Young buds of leafy thalli developed from monospores, × 160.

These *Conchocelis* colonies formed monosporangia and liberated monospores which germinated into normal leafy thalli under short-day conditions.

One of the *Conchocelis* colonies derived from strawberry-like structures produced monosporangia and monospores which gave rise to leafy thalli (2–3 mm. in length) even in long-day conditions. This behavior of the *Conchocelis* phase in long-day conditions (16 hours daily, fluorescent light) had never been observed in our culture *in vitro*. Is this *Conchocelis* phase a special one or a mutant? More work is needed to solve these questions.

The "Concho-monospores" and strawberry-like structures have been observed at temperatures higher than 18° C. and under long-day conditions. It seems that the formation of inflated spherical cells and the strawberry-like structures is affected by photoperiodism and temperature.

The sporangia (SI) produced by *Conchocelis* colonies grown under long-day conditions resemble in function the plantlets that Kornmann used to start his cultures (1960). These sporangia produce spores which germinate into new *Conchocelis* colonies having well developed sporangia under long day, though their germination rate is considerably lower.

In the short-day condition, the spores develop into "plantlets" (ours) having a few or poor filaments (rhizoids). The "plantlets" are morphologically very similar to the sporangia that *Conchocelis* develops in long day but seem to be functionally different.

Although numerous "plantlets" with rhizoids appear in the culture of *P. pseudolinearis* (Plate IV), this type of "plantlet" without rhizoids has not been found in *P. tenera* cultures. The "plantlets" found in our cultures under short-day condi-

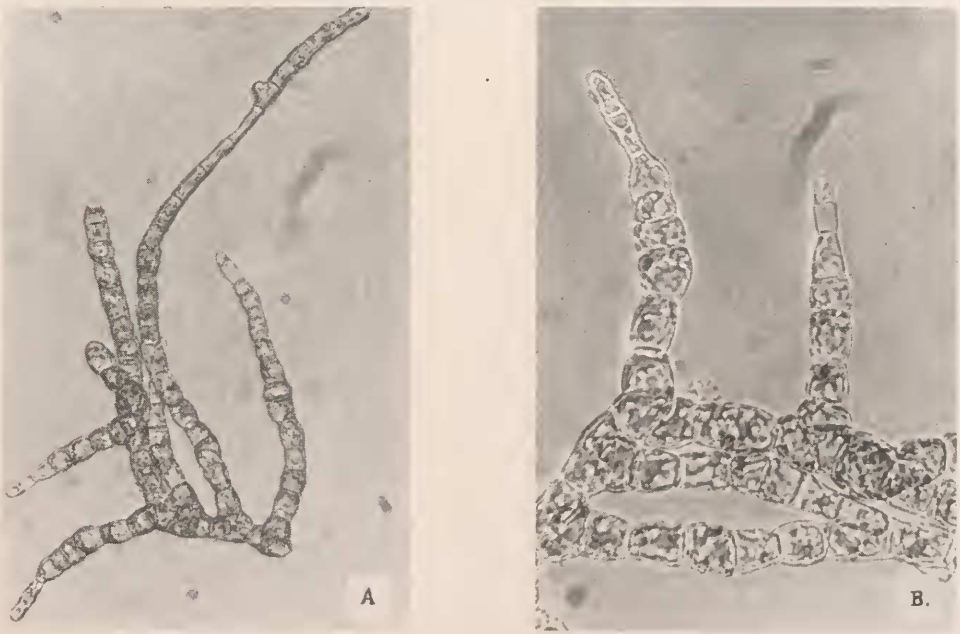

PLATE IV

"Plantlet" without rhizoids of *Porphyra pseudolinearis*. A, × 192; B, × 384.

tions are similar to the "plantlets" described by Drew (1954) in her culture of *P. umbilicalis*.

The blade-like bodies developed in short day (Plate III, F, G) look like young buds of leafy thalli but they do not elongate to more than 1 mm. in length and do not show longitudinal divisions (Plate III, F, G and H).

What are the globular and irregular blade-like bodies (Plate II, C, D and G) developed in long-day conditions, and what roles do they play in the life-cycle? And also, what are the blade-like bodies (Plate III, G) and "massive plantlets" (Plate III, I–O) in short-day conditions? Are they merely abnormal germinations of leafy thalli? The "plantlet" seen in Plate III, O, seems ready to liberate some unknown spores.

The variety of structures created under different light and temperature conditions shows that *P. tenera* has unusual power of adaptation to the environment, and more work is needed to solve some of the problems posed by the growth potencies of *P. tenera*.

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Summary

1. Free-living *Conchocelis* colonies under long-day conditions produce several types of reproductive bodies from which new *Conchocelis* colonies originate, revealing a new part of the life-cycle.

2. Three reproductive structures were observed and described: (a) inflated spherical cells; (b) strawberry-like structures; (c) special "sporangia" (SI).

3. The inflated spherical cells discharge single spores which develop *Conchocelis* colonies.

4. The interior cells of the strawberry-like structures and unicellular spore-like bodies discharged by disintegration of the structure produce germ tubes, and grow into *Conchocelis* colonies.

5. These *Conchocelis* colonies produce monosporangia and liberate the monospores that germinate into normal leafy thalli under short-day conditions. The spherical cells and the strawberry-like structures were produced on *Conchocelis* branches at temperatures higher than 18° C. and under long-day conditions (subdued light).

6. The spores liberated from sporangia (SI) formed under long-day conditions develop into various plantlets: (a) *Conchocelis*-like plants, globular bodies and abnormal blades in long day (14 hours' illumination daily); (b) plantlets having short and root-like filaments, blade-like bodies and massive plantlets in short day (9 hours' illumination daily).

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